

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney's Docket No.: 101195-18

Examiner : A. Chakrabarti  
Group Art Unit : 1655  
Applicant(s) : Timo Hillebrand  
Serial No. : 09/454,740  
Filed : December 6, 1999  
For : Formulations and method for isolating  
nucleic acids from optional complex starting  
materials and subsequent complex gene  
analysis

#20  
Hillebrand  
11

DECLARATION UNDER 37 C.F.R. §1.132

Hon. Commissioner of Patents  
Washington, D.C. 20231

Sir:

I, Timo Hillebrand, hereby declare as follows:

1. I am a named inventor in the above-referenced  
application.

2. I am a German citizen and reside at Berlin;

3. I conducted the experiments set forth in detail for the  
use of the kit in isolating nucleic acids in the presence of

antichaotropic reagents in the attachment hereto, and I submit this declaration in support of the above-referenced application. The 'classical method' as noted at page 1, lines 34-38 of the specification, is detailed herein at paragraph 4 as a comparison study to the above-referenced application.

4. Genomic DNA was isolated from yeasts by resuspending the yeast in 100  $\mu$ l phenol/chloroform/ isoamylalcohol and 100  $\mu$ l lysis buffer, wherein the lysis buffer contains 2% Triton, 1% SDS, 100 mM NaCl, 100 mM Tris-HCl, and 0.1 mM EDTA. Then 0.1 g of glass beads was added to the lysis mixture, which was followed by a vortexing of the lysis mixture. The lysis mixture is then centrifuged and the resulting supernatant containing the isolated genomic DNA is removed. 3 M NaOAc and 250  $\mu$ l are added to the supernatant to precipitate the DNA. Precipitation of the DNA further includes an incubation of the supernatant at -20 °C, followed by centrifuging the supernatant to pellet the DNA, washing the pelleted DNA, and resuspending the DNA in TE or water.

5. The method described in paragraph 4 illustrates an extraction of DNA whereby phenol, chloroform, and ethanol are employed as chemical agents. Further, the glass beads act as physical agents for the mechanical destruction of the yeast

cells, and therefore, are not a binding surface for the genomic DNA. It is noted that the genomic DNA is not bound to any other solid phase in the method described above.

All statements made herein on knowledge are true, and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: November 27, 2001



(Signature)